Influence of acute-phase proteins on erythrocyte aggregation

XIAODUAN WENG, GUY CLOUTIER, RAYMOND BEAULIEU, AND GHISLAINE O. ROEDERER
Laboratories of Biomedical Engineering and Hyperlipidemia and Atherosclerosis, Clinical Research Institute of Montreal and Division of Oncology and Hematology, Hôtel-Dieu of Montreal Hospital, Montreal, Quebec, Canada H2W 1R7

Weng, Xiaoduan, Guy Cloutier, Raymond Beaulieu, and Ghislaine O. Roederer. Influence of acute-phase proteins on erythrocyte aggregation. *Am. J. Physiol.* 271 (*Heart Circ. Physiol.* 40): H2346–H2352, 1996.—With the exception of fibrinogen, immunoglobulins, and albumin, little information is available on the effect of acute-phase proteins on erythrocyte aggregation. The objective of this study was to investigate the effects of haptoglobin (Hp), C-reactive protein (CRP), ceruloplasmin (Cp), α_1-acid glycoprotein (α_1-AGP), and α_1-antitrypsin (α_1-AT) on the aggregation kinetics and shear resistance of erythrocyte aggregates. The plasma concentration of these proteins was measured in 20 healthy individuals and kept unchanged while the concentration of the protein tested was increased. Adding Hp to concentrations between 2.78 and 4.99 g/l resulted in a significant progressive increase in aggregation kinetics compared with controls. An elevation of the shear resistance of the aggregates was found for CRP at a concentration of 0.438 g/l. By an increase in the concentration of Cp from 4.40 to 9.39 g/l, the aggregation kinetics and the adhesive forces between erythrocytes were significantly increased. No effect on erythrocyte aggregation was observed for α_1-AGP, α_1-AT, and Cp at concentrations of 2.85, 3.97, and 2.43 g/l, respectively. The molecular mass of the acute-phase proteins, their configuration, and the presence of specific receptors on the erythrocyte membrane are postulated as possible factors influencing erythrocyte aggregation.

haptoglobin; C-reactive protein; ceruloplasmin; laser light erythroaggregometer

UNDER SLOW FLOW AND STASIS conditions, erythrocytes can aggregate to form rouleaux in the presence of plasma macromolecular proteins. Erythrocyte aggregation and disaggregation are dynamic, reversible, and normal physiological processes. Rouleaux of erythrocytes are normally easily broken down by external forces (e.g., shear stress), and they are formed again when these external forces are reduced or removed. However, under pathological conditions, the formation of compact aggregates characterized by strong intercellular links may induce microcirculatory problems (39) and be a risk factor for cardiovascular and thrombotic diseases (19, 37).

Erythrocyte hyperaggregability has been associated with diabetes (18), dyslipidemia (30), hypertension (31, 41), cardiac and cerebral vascular diseases (23, 38), thromboembolic events (3), carcinomas (15), and inflammation (8, 28). In patients with unstable angina pectoris, abnormal erythrocyte aggregation levels were found to be predictive of subsequent acute myocardial infarction (23). In arterial hypertension, the changes in blood rheology associated with an increase in erythrocyte aggregation were postulated to contribute to the development of left ventricular hypertrophy (31, 41). Increasing erythrocyte aggregation has also been associated with distal angiopathy of the lower limbs in patients with diabetes mellitus (18), and Stoltz and Donner (37) pointed out the importance of rouleaux formation in patients with vascular thrombosis.

Previous studies have shown that some plasma macromolecular proteins can produce major changes in rouleaux formation and erythrocyte aggregation. An increase in plasma fibrinogen (Fb) levels resulted in an elevation of erythrocyte aggregation (26, 28, 29). As summarized by Chien et al. (7), erythrocyte aggregation also depends on α_1- and β_2-globulins. Immunoglobulins (IgG, IgA, and IgM) and IgG fragments accelerated the velocity of aggregate formation (13). The protein α_2-macroglobulin was identified as a globulin inducing erythrocyte aggregation in patients with myeloma (34). In contrast, albumin is considered an inhibiting factor of erythrocyte aggregation (11). Little information is available on the influence of other acute-phase proteins on erythrocyte aggregation.

The term "acute-phase proteins" is defined as a group of plasma glycoproteins synthesized by the liver that increase in concentration in cases of traumatic and inflammatory processes (16). In recent years, some studies demonstrated that these proteins could be increased after tissue injury and in response to a wide variety of clinical conditions. The acute-phase serum proteins of hepatic origin, such as haptoglobin (Hp), C-reactive protein (CRP), ceruloplasmin (Cp), α_1-antitrypsin (α_1-AT), and α_1-acid glycoprotein (α_1-AGP), were found elevated in diabetes (22). A significant elevation of Hp, CRP, and α_1-AGP was reported in a variety of cancers (17, 36). As described by Wang et al. (40), Hp was associated with various cardiac conditions, whereas CRP significantly increased in hypertriglyceridemia (9). Significantly elevated levels of Cp were found in myocardial infarction, diabetes with peripheral vascular disease, and carcinomas (4, 17, 20, 25, 32).

Hypotheses and objectives. Because the concentration of some acute-phase proteins was found to be elevated in many diseases associated with hyper erythrocyte aggregation, it was hypothesized that these proteins may be involved in the mechanisms of erythrocyte aggregation. The present investigation was thus designed to study quantitatively the role of five acute-phase proteins on the aggregation kinetics and the adhesive forces between erythrocytes. The proteins

investigated were Hp, CRP, Cp, α_1-AGP, and α_1-AT. Fb was also studied to compare our results, since its effect on erythrocyte aggregation is well documented. In the experimental design described below, the concentration of the protein under study was increased while the concentration of other plasma proteins was kept constant. This approach was used mainly to simulate physiological and pathological conditions. The possible interaction between acute-phase proteins on erythrocyte aggregation was not addressed in the present study.

MATERIALS AND METHODS

Acute-phase proteins. Hp, CRP, Cp, α_1-AGP, and α_1-AT were purchased from Sigma Chemical (St. Louis, MO); Fb was obtained from Calbiochem-Novabiochem (La Jolla, CA). All proteins were purified from human plasma, and their purities were between 95 and 99%. Hp, α_1-AGP, α_1-AT, and Fb were distributed in powder. CRP was dissolved in a solution of 0.02 M tris(hydroxymethyl)aminomethane (Tris) and 0.25 M sodium chloride (pH 8.0) at a concentration of 2 g/l. Cp was buffered in a solution of 0.25 M sodium chloride and 0.05 M sodium acetate at a concentration of 110 g/l (pH 7.0).

Preparation of samples. Fresh blood was obtained from 20 healthy adult volunteers (22–40 yr, 11 males and 9 females) and anticoagulated with EDTA (1.5 g/l) in standard Vacutainer tubes. All experiments were performed within 4 h after venipuncture. The blood samples were centrifuged (IEC PR-6000) at 1,300 *g* for 10 min, and the hematocrit was adjusted at 0.40 by addition or subtraction of autologous plasma. The hematocrit was measured with a microcentrifuge (Haemofuge, Heraeus Instruments) operating at 14,980 *g* for 10 min.

In a first series of experiments, blood from five subjects was collected and subdivided into seven subsamples of 1.5 ml for each individual. For the first five subsamples, 2 mg of Hp, Cp, α_1-AGP, α_1-AT, and Fb were dissolved separately in plasma and reconstituted with the cellular components of blood. The concentration of the protein added in each subsample was 2.22 g/l of plasma. In another subsample, 0.15 mg of CRP was added, corresponding to a plasma concentration of 0.17 g/l. The last subsample served as the control. Because Cp and CRP were provided dissolved in a saline solution, a small volume of plasma was eliminated and replaced by the protein solution to maintain the hematocrit at 0.40. The objective of this study was to identify proteins affecting erythrocyte aggregation.

According to the results obtained in this study and theoretical considerations, Hp, CRP, and Cp were selected for additional analyses. In a second series of experiments, measurements were repeated on blood samples from five subjects for each protein. Subsamples of blood were prepared for each donor, and different concentrations of the protein were tested. For Hp, concentrations of 1.67, 2.78, and 3.89 g/l were added directly in the plasma of the test subsamples, and another subsample served as the control. Concentrations of 0.11, 0.28, and 0.44 g/l of plasma were added to the test subsamples for CRP; plasma concentrations of 2.50, 4.17, 5.83, 7.50, and 9.17 g/l were used for Cp. As mentioned above, for each test subsample, a volume of plasma was replaced by the protein solution for CRP and CP. However, to avoid the influence of the dilution of the other plasma proteins on erythrocyte aggregation, two control groups were used this time for CRP and Cp. The whole blood collected from each individual served as one control group, and the other control group was prepared by replacing a volume of plasma by a solution of 0.02 M Tris and 0.25 M sodium chloride for CRP and a solution of 0.25 M sodium chloride and 0.05 M sodium acetate for Cp. The volume of the solutions replaced in the control subsamples corresponded to the volume of the protein solution added in the test subsamples. No significant reduction of the plasma concentration of Cp and CRP (2nd decimal for Cp and 3rd decimal for CRP) was noted between protein groups and control solution groups.

Measurements of erythrocyte aggregation. In both series of experiments described above, the blood subsamples were incubated in a warm bath (37°C) for 10 min before measurement of erythrocyte aggregability. For Cp, the incubation was performed in a dark environment because Cp is light sensitive. Erythrocyte aggregation measurements were performed at a constant hematocrit (0.40) with an erythroaggregometer based on a Couette flow arrangement (Regulest, Florange, France). A laser diode (Hitachi, HL7801G) providing a radiation at 780 nm was used as the light source. Parameters of erythrocyte aggregation were derived from the analysis of the variations in light intensity of the signal scattered by blood. Each blood sample (1.5 ml) was put into a chamber between two coaxial cylinders, and the outside cylinder was rotated to provide different shear rate conditions. All measurements were performed at 37°C by controlling the temperature in the blood chamber with circulating water.

Two series of measurements were performed. In the first series, the blood sample was sheared for 10 s at 550 s^{-1} to provide rouleaux disruption and erythrocyte orientation with the flow. After abrupt cessation of the rotation, the variation of the scattered light intensity was recorded during the rouleaux formation process. The instrument provided the following important parameters on erythrocyte aggregation kinetics: t_A and S_{10}, corresponding to the primary aggregation time and a mean kinetic index at 10 s, respectively. The parameter t_A was obtained from the inverse of the slope of the scattered light intensity variation between 0.3 and 1.9 s after stoppage of the flow rotation. The index S_{10} was calculated as the ratio of the area above the light intensity curve during the first 10 s after flow stoppage to the total area within the same period of time. For the second series of measurements, the blood sample was sheared at 96 different levels from 6 to 720 s^{-1}. A curve describing the scattered light intensity as a function of the shear rate was obtained for each blood sample. The intensity increased to reach a maximum and then decayed. The partial dissociation threshold (γ_D) was obtained from the intersection of the regression line computed for shear rates below 20 s^{-1} and the horizontal line intercepting the maximum of the scattered light intensity. The total dissociation threshold (γ_S) corresponded to the shear rate at the maximum scattered light intensity. These last two parameters provide information on the adhesive forces between erythrocytes.

Determination of plasma acute-phase protein concentrations. For all control samples collected from the 20 healthy subjects, the original concentration of all acute-phase proteins in the plasma was determined by the immunonephelometric method for Hp, Cp, α_1-AGP, and α_1-AT, the latex agglutination technique (semiquantitative) for CRP, and the Von Clauss approach for Fb. All measurements were performed at the Hôtel-Dieu of Montreal Hospital.

Statistical analyses. All results were expressed as means ± SD. One-way repeated-measures analysis of variance with Bonferroni's method for multiple comparisons (SigmaStat statistical software, Jandel Scientific) was used to assess the differences between each blood subsample. A significance level of 5% was used in all analyses.

Table 1. *Measured and tested plasma concentrations of acute-phase proteins in healthy control subjects and range of values reported in literature for normal subjects*

Acute-Phase Protein	Plasma Concentrations, g/l		
	Measured	Tested	Reported*
Hp	1.26 ± 0.41	2.78 ± 0.40 to 4.99 ± 0.40	1.00–3.00
Cp	0.23 ± 0.03	2.43 ± 0.02 to 9.39 ± 0.03	0.15–0.60
α_1-AGP	0.66 ± 0.17	2.85 ± 0.16	0.55–1.40
α_1-AT	1.84 ± 0.22	3.97 ± 0.08	2.00–4.00
Fb	2.51 ± 0.35	4.59 ± 0.22	2.00–4.50
CRP	20/20 negative (<0.006)	0.116 to 0.438	<0.01

Values are means ± SD for measured and tested plasma concentrations except for C-reactive protein; $n = 20$ subjects; $n = 5$ for each protein. Note that plasma concentrations tested for each protein are obtained by adding plasma concentration measured for each group of 5 subjects to concentrations added in plasma (see MATERIALS AND METHODS). Hp, haptoglobin; Cp, ceruloplasmin; α_1-AGP, α_1-acid glycoprotein; α_1-AT, α_1-antitrypsin; Fb, fibrinogen; CRP, C-reactive protein. *From Ref. 27.

RESULTS

Plasma concentrations of acute-phase proteins. The original plasma concentrations of each acute-phase protein in the control samples of the 20 healthy individuals and the range of concentrations tested are presented in Table 1. With the exception of α_1-AT (normal range between 2.0 and 4.0 g/l) and Fb (normal range between 2.0 and 4.5 g/l), the concentrations tested were well above the ranges of variation reported in normal subjects (27). As explained later in DISCUSSION, the concentrations tested for each protein were selected to be within the range of increase found in diseases characterized by an elevated level of erythrocyte aggregation.

Influence of acute-phase proteins on erythrocyte aggregation. As shown in Table 2, Hp significantly ($P < 0.05$) decreased t_A, increased S_{10}, and had no effect on the adhesive forces between erythrocytes (γ_D and γ_S) compared with the control samples. The variations of t_A and S_{10} correspond to an elevation of the velocity of rouleaux formation. CRP had an effect on erythrocyte aggregation, whereas Cp, α_1-AGP, and α_1-AT had very little influence at the concentrations tested. A significant effect of Fb on the aggregation kinetics (S_{10}) and adhesive forces (γ_S) were observed. On the basis of our results, S_{10} and γ_S were the most sensitive parameters to variations in the aggregation kinetics and adhesive forces between erythrocytes, respectively.

Effects of varying concentrations of Hp, CRP, and Cp. From the above results, Hp and CRP can produce variations in the aggregation of erythrocytes. Even though no variation was found for Cp at an added concentration of 2.22 g/l (mean plasma concentration of 2.43 g/l), the effect of varying the relative quantity of this protein was tested considering its large molecular mass (see DISCUSSION for more explanations). As shown in Fig. 1, a significant progressive increase in the aggregation kinetics (from reduction of t_A and increase in S_{10}) was observed for mean plasma concentrations of Hp between 2.78 and 4.99 g/l. No statistically significant variation in the adhesive forces (γ_D and γ_S) was found, even at the mean concentration of 4.99 g/l.

Figure 2 presents the results obtained by adding solutions with three different concentrations of CRP. The effect of CRP on the aggregation kinetics and γ_D was close to that obtained with the control solution. However, compared with the control solution, a significant increase in γ_S was observed at a mean plasma concentration of 0.438 g/l. Figure 3 summarizes the results obtained by adding solutions with five different concentrations of Cp. Compared with the control solutions, statistically significant increases of the aggregation kinetics (from t_A and S_{10}) were found for mean plasma concentrations of 4.40 g/l and higher. The shear resistance of the aggregates (from γ_D and γ_S) was increased at concentrations of 7.72 and 9.39 g/l.

DISCUSSION

Pathological significance of concentrations of acute-phase proteins tested. As summarized by Koj (16), at least five classes of plasma acute-phase proteins can be defined on the basis of their relative changes in concentration after injury. CRP is a very strong acute-phase reactant usually increasing by 20- to 100-fold (*class A*). An increase in the concentration of CRP up to 1,000-fold a few hours after inflammation, burns, and other trauma was reported (27). According to Koj (16), Hp, Fb, and α_1-AGP are strong acute-phase reactants (*class B*), usually increasing by 100–400%. Cp is a weak acute-phase reactant (*class C*), increasing by 20–60%, whereas α_1-AT is a negative acute-phase reactant (*class*

Table 2. *Influence of acute-phase proteins on erythrocyte aggregation*

	Control	Hp	CRP	Cp	α_1-AGP	α_1-AT	Fb
t_A, s	2.9 ± 0.9	2.3 ± 0.5*	3.3 ± 1.0	3.0 ± 1.0	3.0 ± 1.0	2.9 ± 0.9	2.5 ± 0.6
S_{10}	23 ± 4.4	26 ± 3.5*	21 ± 4.1†	22 ± 4.1	22 ± 4.5	23 ± 3.9	25 ± 3.5*
γ_D, s^{-1}	55 ± 15	51 ± 12	54 ± 17	56 ± 15	54 ± 15	51 ± 14	62 ± 15
γ_S, s^{-1}	116 ± 24	114 ± 24	133 ± 32†	124 ± 23	120 ± 29	120 ± 28	174 ± 52*

Values are means ± SD; $n = 5$. See MATERIALS AND METHODS for plasma concentrations tested in this part of the study. t_A, Primary aggregation time; S_{10}, mean kinetic index at 10 s; γ_D, partial dissociation threshold; γ_S, total dissociation threshold. *$P < 0.05$; statistical comparisons performed between protein groups and control group. †$P < 0.05$. For CRP and CP, it was believed in the 1st phase of the study that the effect of adding a small volume of saline solution would have little influence on erythrocyte aggregation indexes. It was observed later during the 2nd phase of the study that this postulate was incorrect. Consequently, statistically significant results presented here for CRP should be interpreted with caution.

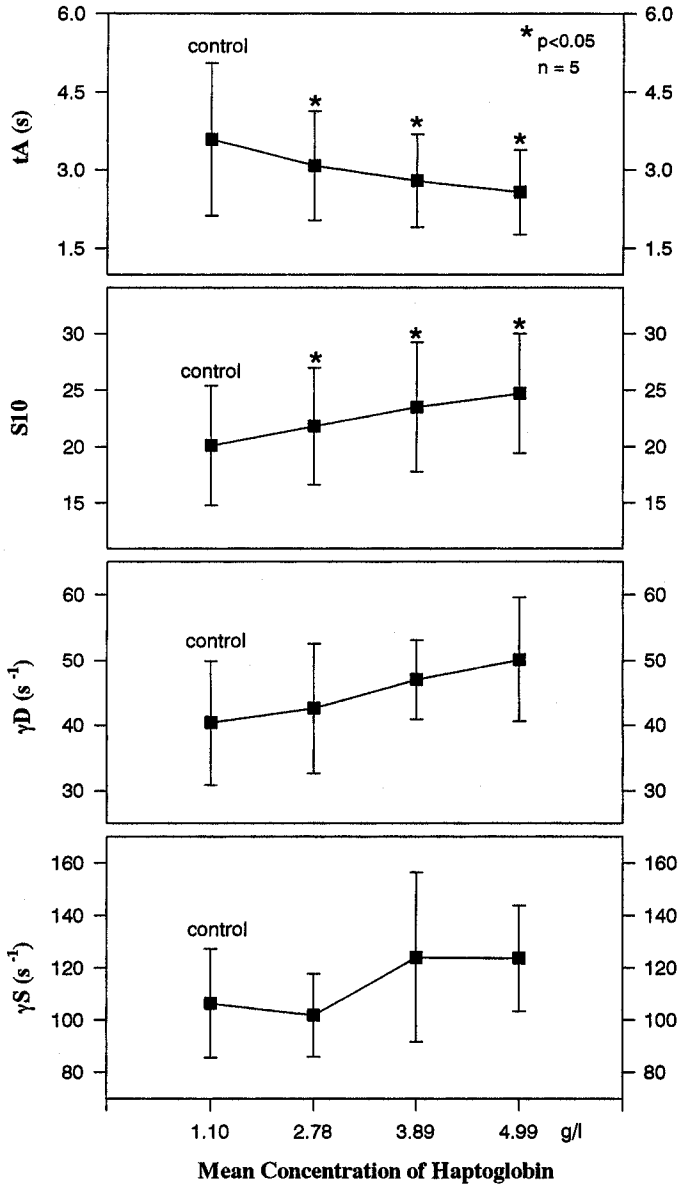

Fig. 1. Influence of different plasma concentrations of haptoglobin (Hp) on aggregation kinetics [primary aggregation time (t_A) and mean kinetic index at 10 s (S_{10})] and shear resistance [partial dissociation threshold(γ_D) and total dissociation threshold (γ_S)] of erythrocyte aggregates. Statistical comparisons were performed between protein group at different concentrations and control group to which no Hp was added.

E), usually decreasing by 20–60% in response to trauma (16).[1] However, for other specific diseases, different ranges of variation of the concentration of these acute-phase proteins were reported. A raise of CRP by only 94% was found in hypertriglyceridemia (9). In diabetes, the concentrations of Hp, α_1-AGP, Cp, and α_1-AT were increased by 43, 23, 14, and 12%, respectively, compared with healthy control subjects (22). An increase in the concentration of Cp by 36–70% was observed in carcinomas, myocardial infarction, and peripheral vascular disease (4, 17, 20, 25, 32).

In summary, the changes in the acute-phase protein concentration in diseases related to an elevated level of erythrocyte aggregation are broad, ranging from 43 to 400% for Hp, 14 to 70% for Cp, 23 to 400% for α_1-AGP, −60 to 12% for α_1-AT, 100 to 400% for Fb, and 94% to 1,000-fold for CRP. In the present study, according to Table 1, the concentration of Hp was increased by 121–296% compared with controls, which is within the range of values found after injury (16). The concentration of CRP was raised by ~19- to 73-fold, which is also within the range of values reported by Koj (16). Cp was increased by 10.6- to 40.8-fold, well above pathological concentrations reported in humans (4, 16, 22). It was

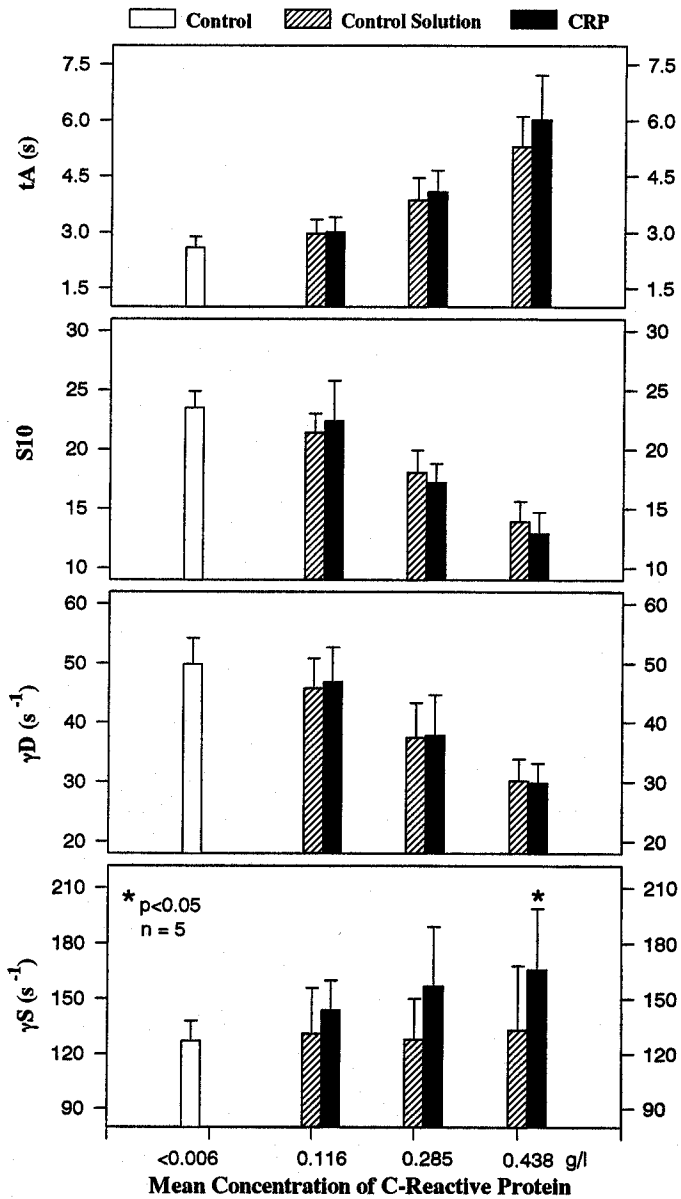

Fig. 2. Influence of different plasma concentrations of C-reactive protein (CRP) on t_A, S_{10}, γ_D, and γ_S of erythrocyte aggregates. Statistical comparisons were performed between all 7 experimental groups. Only *P* values between protein groups and control solution groups are given.

[1] According to Koj (16), *class D* represents neutral proteins. No such type of protein was tested in the present study.

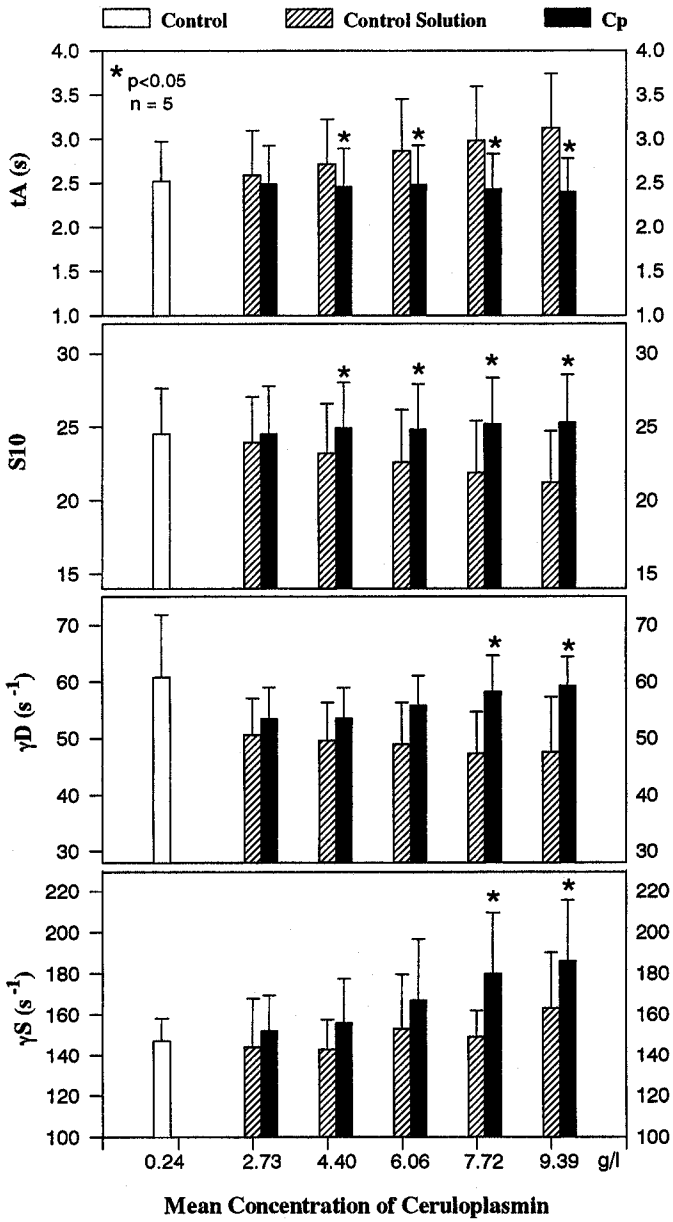

Fig. 3. Influence of different plasma concentrations of ceruloplasmin (Cp) on t_A, S_{10}, γ_D, and γ_S of erythrocyte aggregates. Statistical comparisons were performed between all 11 experimental groups. Only P values between protein groups and control solution groups are given.

observed that α_1-AGP had no marked influence on erythrocyte aggregation despite the use of a plasma concentration (an increase of 332%) within values measured after trauma (16). A similar observation was noted for α_1-AT at a concentration (an increase of 116%) higher than the values found in diabetes (22). The plasma concentration increase in Fb (83%) was conservative, since 100–400% variations were reported during acute-phase responses (16). Even at this relatively low concentration, Fb had a marked influence on the aggregation kinetics and adhesive forces between erythrocytes.

As reported before, Cp concentrations well above pathological levels reported in humans were used in the present study (mean plasma concentrations between 2.43 and 9.39 g/l). It is interesting to observe that a plasma concentration of Cp of 6.06 ± 0.74 g/l was reported by Okumura et al. (24) in normal adult equine blood. Because horse blood has a very high erythrocyte aggregation tendency (10), the possibility that Cp may intervene in the mechanism of erythrocyte aggregation was postulated and verified by use of mean concentrations up to 9.39 g/l.

Theoretical models of erythrocyte aggregation. Erythrocyte aggregation as well as other types of membrane adhesion processes can be classified into two categories: the specific and nonspecific binding. The specific binding occurs via the interaction of suspended macromolecules with their specific receptors on the erythrocyte membrane. For nonspecific erythrocyte adhesion mechanisms, two major theoretical models were proposed (1). The first theory based on macromolecular bridging is well accepted for describing the process of erythrocyte rouleaux formation. It is based on the surface adsorption of macromolecules to form a bridging configuration between adjacent erythrocytes (2, 5, 6). Van der Waals forces, hydrogen bonds, or electrostatic attractions are believed to favor the adsorption of macromolecules (5). The other theory suggests that the aggregation is induced by the macromolecular depletion from the membrane surface (12, 14). According to the former nonspecific adhesion theory, the degree of erythrocyte aggregation is mainly dependent on the molecular mass of the macromolecules and the surface adsorption (6). On the basis of this theory, polymers and plasma proteins with a large molecular mass insert between adjacent erythrocytes, increase the intercellular distance, and induce erythrocyte aggregation by decreasing the electrostatic repulsive forces between erythrocytes. The enhanced aggregation can also be explained, according to this theory, by an increase in the adsorption area on the cell surface due to the larger size of the macromolecules (6). In the latter nonspecific adhesion theory, the aggregation is independent of both the molecular mass and the surface adsorption. The attraction of colloidal particles producing the aggregation is induced by variations in the surface energy and differences in osmotic pressure due to a profile of polymer concentrations existing in the suspending medium between the neighboring surfaces (12, 14).

Hypotheses on role of Hp, CRP, and Cp on erythrocyte aggregation. Our data demonstrated that the effectiveness of the acute-phase proteins in affecting erythrocyte aggregation varied with the molecular mass. The acute-phase proteins with a large molecular mass (27) such as Hp (86–400 kDa), CRP (105 kDa), Cp (132 kDa), and Fb (340 kDa) exhibited remarkable effects on erythrocytes. In contrast, the acute-phase proteins with a small molecular mass, such as α_1-AGP (40 kDa) and α_1-AT (54 kDa), played no significant role on erythrocyte aggregation. Hence, our findings seem to be in agreement with the nonspecific adhesion theory of macromolecular bridging.

The configuration of the protein may also intervene in the mechanisms of erythrocyte aggregation. The

globular Fb with a molecular mass of 680 kDa did not induce erythrocyte aggregation (35), whereas fibrous Fb (length of ~45 nm), having a molecular mass of 340 kDa, markedly accelerated the erythrocyte aggregation (21). It is the fibrous Fb that was used in the present study. Hp being a four-chain glycoprotein with a polymorphic structure (27) may favor the surface adsorption as well as the macromolecular bridging between membranes of adjacent erythrocytes, as reflected by its effect on the aggregation kinetics. Cp is a sky-blue copper transport α_2-glycoprotein with a signal polypeptide chain consisting of 1,046 amino acids in an internally triplicated structure that contains six domains (27). Specific receptors on the erythrocyte membrane were identified for this protein (33). Two mechanisms related to the structure of Cp as well as its specific adhesion receptors on the erythrocyte membrane may explain the influence of this protein on the aggregation kinetics and shear resistance of the aggregates. The cyclic pentamer structure of CRP and the large area of surface adsorption due to the high molecular mass of this protein may intervene in strengthening the adhesives forces between erythrocytes.

In addition to the above factors affecting erythrocyte aggregation, the concentration of the protein was shown to play an important role in the mechanism of erythrocyte aggregation. This could be explained by a rising number of bridges in the theory of macromolecular bridging, by variations in the surface energy and osmotic pressure in the theory of depletion, and by a rising number of binding receptors in the specific adhesion theory.

In conclusion, the present study demonstrated that an abnormally high level of Hp induced a significant increase in the reversible erythrocyte rouleaux formation. This effect increased with rising protein concentrations. On the other hand, no marked change in the adhesive forces between erythrocytes was observed, even at the highest mean plasma concentration tested (4.99 g/l). CRP significantly increased the adhesive forces of the aggregates for a mean plasma concentration of 0.438 g/l. The addition of Cp significantly enhanced the erythrocyte aggregate formation for mean plasma concentrations of 4.40 g/l and higher, and the shear resistance of the aggregates for mean concentrations of 7.72 and 9.39 g/l. It is important to note, however, that these last concentrations are well above the levels found in human. In summary, the present study clearly demonstrated that acute-phase proteins other than Fb and immunoglobulins can affect erythrocyte aggregation. It is possible that Hp and CRP may be involved in human pathological conditions characterized by an elevated level of erythrocyte aggregation. The high erythrocyte aggregation in some mammals may be explained by a high concentration of Cp.

This work was supported by a research scholarship from the Fonds de la Recherche en Santé du Québec (G. Cloutier) and by grants from the Whitaker Foundation, US, the Medical Research Council of Canada (MA-12491), and the Heart and Stroke Foundation of Quebec.

Address for reprint requests: G. Cloutier, Lab. of Biomedical Engineering, Clinical Research Institute of Montreal, 110 Ave. des Pins Ouest, Montreal, PQ, Canada H2W 1R7 (E-mail address: Cloutig@ircm.umontreal.ca).

Received 12 February 1996; accepted in final form 6 May 1996.

REFERENCES

1. **Brooks, D. E.** Current models of red blood cell aggregation (Abstract). *Biorheology* 32: 103, 1995.
2. **Brooks, D. E., and G. V. F. Seaman.** The effect of neutral polymers on the electrokinetic potential of cells and other charged particles. I. Models for the zeta potential increase. *J. Colloid Interface Sci.* 43: 670–686, 1973.
3. **Chabanel, A., M. H. Horellou, J. Conard, and M. M. Samama.** Red blood cell aggregability in patients with a history of leg vein thrombosis: influence of post-thrombotic treatment. *Br. J. Haematol.* 88: 174–179, 1994.
4. **Chan, A., F. Wong, and M. Arumanayagam.** Serum ultrafiltrable copper, total copper and caeruloplasmin concentrations in gynaecological carcinomas. *Ann. Clin. Biochem.* 30: 545–549, 1993.
5. **Chien, S.** Biophysical behavior of red cells in suspensions. In: *The Red Blood Cell.* New York: Academic, 1975, vol. II, chapt. 26, p. 1032–1133.
6. **Chien, S., and K. M. Jan.** Ultrastructural basis of the mechanism of rouleaux formation. *Microvasc. Res.* 5: 155–166, 1973.
7. **Chien, S., S. Usami, R. J. Dellenback, and C. A. Bryant.** Comparative hemorheology—hematological implications of species differences in blood viscosity. *Biorheology* 8: 35–37, 1971.
8. **Copley, A. L.** Agrégation et désagrégation érythrocytaires. In: *Hémorhéologie et agrégation érythrocytaire.* Paris: Éditions Méd. Int., 1986, p. 9–21.
9. **Crook, M., S. Haq, M. Haq, and P. Tutt.** The relationship of serum total sialic acid with serum acute phase proteins and lipoprotein (a) in patients with severe hypertriglycedidaemia. *Eur. J. Clin. Invest.* 24: 179–181, 1994.
10. **Dintenfass, L., and F. L. Liao.** Plasma and blood viscosities, and aggregation of red cells in racehorses. *Clin. Phys. Physiol. Meas.* 3: 293–301, 1982.
11. **Donner, M., P. Mills, and J. F. Stoltz.** Influence des protéines plasmatiques sur l'agrégation érythrocytaire. In: *Hémorhéologie et agrégation érythrocytaire.* Paris: Éditions Méd. Int., 1988, vol. 2, p. 3–8.
12. **Evans, E., and D. Needham.** Attraction between lipid bilayer membranes in concentrated solutions of nonadsorbing polymers: comparison of mean-field theory with measurements of adhesion energy. *Macromolecules* 21: 1822–1831, 1988.
13. **Imaizumi, K., and T. Shiga.** Effect of immunoglobulins and IgG-fragments on the human erythrocyte aggregation, studied by a rheoscope combined with image analyzer. *Biorheology* 20: 569–577, 1983.
14. **Joanny, J. F., L. Leibler, and P. G. De Gennes.** Effects of polymer solutions on colloid stability. *J. Polymer Sci.* 17: 1073–1084, 1979.
15. **Khan, M. M., R. R. Puniyani, N. G. Huilgol, M. A. Hussain, and G. G. Ranade.** Hemorheological profiles in cancer patients. *Clin. Hemorheol.* 15: 37–44, 1995.
16. **Koj, A.** Metabolic studies of acute-phase proteins. In: *Pathophysiology of Plasma Protein Metabolism.* New York: Plenum, 1984, chapt. 11, p. 221–248.
17. **Krecicki, T., and M. Leluk.** Acute phase reactant proteins—an aid to monitoring surgical treatment of laryngeal carcinoma. *J. Laryngol. Otol.* 106: 613–615, 1992.
18. **Le Devehat, C., M. Vimeux, G. Bondoux, and T. Khodabandehlou.** Red blood cell aggregation in diabetes mellitus. *Int. Angiol.* 9: 11–15, 1990.
19. **Lowe, G. D. O., and C. D. Forbes.** Rheology of cardiovascular disease. In: *Clinical Blood Rheology.* Boca Raton, FL: CRC, 1988, vol. II, chapt. 13, p. 113–140.
20. **MacRury, S. M., D. Gordon, R. Wilson, H. Bradley, C. G. Gemmel, J. R. Paterson, A. G. Rumley, and A. C. MacCuish.** A comparison of different methods of assessing free radical activity in type 2 diabetes and peripheral vascular disease. *Diabetic Med.* 10: 331–335, 1993.
21. **Maeda, N., K. Imaizumi, M. Sekiya, and T. Shiga.** Rheological characteristics of desialylated erythrocytes in relation to

fibrinogen-induced aggregation. *Biochim. Biophys. Acta* 776: 151–158, 1984.

22. **McMillan, D. E.** Increased levels of acute-phase serum proteins in diabetes. *Metab. Clin. Exp.* 38: 1042–1046, 1989.
23. **Neumann, F. J., H. A. Katus, E. Hoberg, P. Roebruck, M. Braun, H. M. Haupt, H. Tillmanns, and W. Kübler.** Increased plasma viscosity and erythrocyte aggregation: indicators of an unfavourable clinical outcome in patients with unstable angina pectoris. *Br. Heart J.* 66: 425–430, 1991.
24. **Okumura, M., T. Fujinaga, K. Yamashita, N. Tsunoda, and S. Mizuno.** Isolation, characterization, and quantitative analysis of ceruloplasmin from horses. *Am. J. Vet. Res.* 52: 1979–1985, 1991.
25. **Ozyilkan, O., E. Baltali, E. Ozyilkan, G. Tekuzman, A. Kars, and D. Firat.** Ceruloplasmin level in women with breast disease. Preliminary results. *Acta Oncol.* 31: 843–846, 1992.
26. **Potron, G., P. Nguyên, and B. Pignon.** Fibrinogen, arterial risk factor, in clinical practice. *Clin. Hemorheol.* 14: 739–767, 1994.
27. **Putnam, F. W.** *The Plasma Proteins Structure, Function, and Genetic Control.* New York: Academic, 1984, p. 1–400.
28. **Rampling, M. W.** Red cell aggregation and yield stress. In: *Clinical Blood Rheology.* Boca Raton, FL: CRC, 1988, vol. I, chapt. 3, p. 45–64.
29. **Rampling, M. W., P. Whittingstall, and O. Linderkamp.** The effects of fibrinogen and its plasmin degradation products on the rheology of erythrocyte suspensions. *Clin. Hemorheol.* 4: 533–543, 1984.
30. **Razavian, S. M., V. Atger, G. M. Cambillau, M. Del-Pino, A. C. Simon, N. Moatti, and J. Levenson.** Influence of HDL subfractions on erythrocyte aggregation in hypercholesterolemic men. *Arterioscler. Thromb.* 14: 361–366, 1994.
31. **Razavian, S. M., M. Del Pino, A. Simon, and J. Levenson.** Increase in erythrocyte disaggregation shear stress in hypertension. *Hypertension Dallas* 20: 247–252, 1992.
32. **Reunanen, A., P. Knekt, and R. K. Aaran.** Serum ceruloplasmin level and the risk of myocardial infarction and stroke. *Am. J. Epidemiol.* 136: 1082–1090, 1992.
33. **Saenko, E. L., and A. I. Yaropolov.** Studies on receptor interaction of ceruloplasmin with human red blood cells. *Biochem. Int.* 20: 215–225, 1990.
34. **Schmid-Schönbein, H., G. Gallasch, E. Volger, and H. J. Klose.** Microrheology and protein chemistry of pathological red cell aggregation (blood sludge) studied in vitro. *Biorheology* 10: 213–227, 1973.
35. **Sewchand, L. S., M. A. Masri, O. G. Fritz, N. G. Boyd, and S. Rowlands.** Fibrinogen and the ultralong-range interaction of human erythrocytes. *Cell Biophys.* 6: 215–221, 1984.
36. **Staal-van den Brekel, A. J., A. M. W. J. Schols, G. P. M. ten Velde, W. A. Buurman, and E. F. M. Wouters.** Analysis of the energy balance in lung cancer patients. *Cancer Res.* 54: 6430–6433, 1994.
37. **Stoltz, J. F., and M. Donner.** Hemorheology: importance of erythrocyte aggregation. *Clin. Hemorheol.* 7: 15–23, 1987.
38. **Tanahashi, N., F. Gotoh, M. Tomita, T. Shinohara, Y. Terayama, B. Mihara, K. Ohta, and M. Nara.** Enhanced erythrocyte aggregability in occlusive cerebrovascular disease. *Stroke* 20: 1202–1207, 1989.
39. **Vicaut, E., X. Hou, L. Decuypère, A. Taccoen, and M. Duvelleroy.** Red blood cell aggregation and microcirculation in rat cremaster muscle. *Int. J. Microcirc.* 14: 14–21, 1994.
40. **Wang, M. J., A. Chao, C. H. Huang, C. H. Tsai, F. Y. Lin, S. S. Wang, C. C. Liu, and S. H. Chu.** Hyperbilirubinemia after cardiac operation: incidence, risk factors, and clinical significance. *J. Thorac. Cardiovasc. Surg.* 108: 429–436, 1994.
41. **Zannad, F., P. Voisin, F. Brunotte, J. F. Bruntz, J. F. Stoltz, and J. M. Gilgenkrantz.** Haemorheological abnormalities in arterial hypertension and their relation to cardiac hypertrophy. *J. Hypertens.* 6: 293–297, 1988.